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Validation of a pH gradient-based ion-exchange chromatography method for high-resolution monoclonal antibody charge variant separations

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ABSTRACT

Ion-exchange chromatography is widely used for profiling the charge heterogeneity of proteins, including monoclonal antibodies. Despite good resolving power and robustness, ionic strength-based ion-exchange separations are product-specific and time-consuming to develop. We have previously reported a novel pH-based separation of proteins by cation exchange chromatography that was multi-product, high-resolution, and robust against variations in sample matrix salt concentration and pH. In this study, a pH gradient-based separation method using cation exchange chromatography was evaluated in a mock validation. This method was shown to be robust for monoclonal antibodies and suitable for its intended purpose of charge heterogeneity analysis. Simple mixtures of defined buffer components were used to generate the pH gradients that separated closely related antibody species. Validation characteristics, such as precision and linearity, were evaluated. Robustness to changes in protein load, buffer pH and column oven temperature was demonstrated. The stability-indicating capability of this method was determined using thermally stressed antibody samples. In addition, intermediate precision was demonstrated using multiple instruments, multiple analysts, multiple column lots, and different column manufacturers. Finally, the precision for this method was compared to conventional ion-exchange chromatography and imaged capillary isoelectric focusing. These results demonstrate the superior precision and robustness of this multi-product method, which can be used for the high-throughput evaluation of in-process and final product samples.

1. Introduction

Monoclonal antibodies (mAbs) are a very important class of therapeutic proteins in biotechnology, and have been developed to treat a variety of indications to fulfill significant unmet medical needs [1]. Monoclonal antibodies are generally target-specific and well tolerated with a relatively long half-life, contributing to the success of the molecule class for drug development. Of the classes of immunoglobulins, IgG1 is the most commonly used immunoglobulin used for pharmaceutical and biomedical purposes [2,3].

Protein heterogeneity of monoclonal antibodies is monitored as part of the ongoing control system that ensures product quality and consistency [4–6]. Monoclonal antibodies are susceptible to chemical or enzymatic modification, particularly at sites that are exposed to the protein–liquid interface. Product heterogeneity can be caused by C-terminal processing of lysine residues [7–9], deamidation [10,11], glycation (nonenzymatic glucose addition) [12], amino acid sequence variations [8], and noncovalent complexes [13].

Monoclonal IgG antibodies are typically characterized by a variety of orthogonal analytical and biochemical methods, including ion-exchange chromatography and isoelectric focusing [9]. Ion-exchange chromatography (IEC) has been a platform for monoclonal antibody purification and characterization for many years [14], with IEC being a typical component in antibody recovery and characterization systems. IEC separates proteins based on differences in the surface charge of the molecules, with separation being dictated by the protein interaction with the stationary phase. While cation exchange chromatography has been called the gold standard for charge sensitive antibody analysis [15], method parameters, such as column type, mobile phase pH, and salt concentration gradient, often need to be optimized for each individual antibody. Isoelectric focusing separation methods, in either capillary or slab gel format, have been widely used due to the multi-product nature of the separation conditions. The proteins are separated by focusing the proteins in a matrix where the pH of the medium changes as a function of position. One particular methodology, imaging capillary isoelectric focusing (icIEF), has recently been developed for charge heterogeneity analysis of monoclonal antibodies [16].

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Despite some advantages over conventional IEC, such as relatively shorter method development time, the implementation of icIEF in a commercial control system entails significant investment, as specialized equipment as well as vendors for consumables is limited. Recently, a chromatofocusing method, which combines the resolving power of isoelectric focusing and the flexibility and simplicity of ion-exchange chromatography, was reported for routine analysis of monoclonal antibody charge species that employs a linear pH gradient formed by external mixing of two phosphate buffers of different pH [17]. The robustness of this chromatofocusing method was demonstrated using a single mAb, and this method compared favorably to conventional salt-gradient IEC [17].

Before an analytical method can be incorporated into a quality control system, it must first be demonstrated that it is suitable for its intended purpose. Guidelines for the validation of analytical methods have been published in the United States Pharmacopeia [18], by the US Food and Drug Administration (FDA) [19,20], and in published reviews [21]. The guidelines published by the International Conference on Harmonization (ICH) have established a uniform understanding of the performance characteristics which are evaluated in the course of validation [22]. The subset of performance characteristics which require investigation in the course of validation, as well as the strategy for designing appropriate experiments, are based upon the intended purpose of the analytical method. Thus, different validation requirements are outlined by the ICH guidelines for assay, impurity and identity methods [22]. The validation of a stability-indicating method requires analyses of stressed samples in order to demonstrate that the method is suitable [21]. International Conference on Harmonization (ICH) guidelines have established requirements for the validation of stability-indicating methods and clarify requirements for stress studies and robustness studies.

We have previously reported a novel pH-based separation of proteins by cation exchange chromatography (pH-IEC) that was multi-product, high-resolution, and robust against variations in sample matrix salt concentration and pH [23]. Simple mixtures of defined buffer components were used to generate the pH gradients that separate closely related antibody species. This method separated monoclonal antibody species with a wide range of isoelectric points via a complex retention mechanism, combining both ionic-strength and pH. The multi-product aspect of this method translates into much less method development time for new IgG molecules. In addition, the ability of the method to assess charge heterogeneity at a wide range of sample matrix salt concentrations and pH indicates the suitability of the method for use in evaluating in-process samples. Despite these advantages, this multi-product pH gradient-based ion-exchange chromatography method had not yet been validated prior to this work, which is necessary for the transfer of an analytical method to a quality control environment.

We report herein the results of a mock validation of a pH gradient-based ion-exchange chromatography method for evaluating charge heterogeneity of monoclonal antibodies. The validation was deemed a mock validation as it was done as proof of concept rather than strictly for regulatory purposes, which require specific criteria be set in advance and achieved for precision and accuracy [20]. Robustness against changes in protein load, buffer pH and column oven temperature is demonstrated. The stability-indicating capability of this method is determined using thermally stressed antibody samples. In addition, intermediate precision is demonstrated using multiple instruments, multiple analysts, multiple column lots, and different column manufacturers. These results demonstrate the precision, robustness and applicability of this multi-product method, which can be used for the high-throughput evaluation of in-process and final product samples.

2. Experimental

2.1. Instrumentation

Three types of liquid chromatographs were used during this work: an Ultimate 3000 X2 (dual channel) bio-compatible liquid chromatograph (Dionex, Sunnyvale, CA), a Waters 2796 bio-compatible liquid chromatograph (Waters, Milford, MA) and an Agilent 1100 liquid chromatograph (Agilent, Santa Clara, CA), each equipped with an autosampler with sample temperature control capability and a thermal compartment to enclose the column. The Dionex Ultimate 3000 X2 chromatograph included dual ternary low pressure micro-gradient pumps and a four channel UV–Vis detector. The Waters 2796 chromatograph included a quaternary pump and a dual wavelength detector (Waters 2487). The Agilent 1100 chromatograph included a high pressure gradient binary pump and a multiple wavelength detector.

Instrument control, data acquisition and compilation of results for all HPLCs were performed using Dionex Chromeleon software, version 6.8.

2.2. Chemicals and columns

Piperazine dihydrochloride hydrate and imidazole were Fluka brand reagents. All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise indicated.

Monoclonal antibodies used in this work were produced by Genentech (South San Francisco, CA). The mAb standard used throughout the study had a pI value of 7.4, which was determined using capillary isoelectric focusing. Thermally stressed samples were produced by incubating the antibody at 40 °C for 4 weeks.

Two types of ion-exchange of columns were used in this study. ProPac WCX-10 cation exchange columns were obtained from Dionex (Sunnyvale, CA). ProPac columns used in this study were 250 mm × 4.0 mm, 10 μm. To assess method variability between different column manufacturers, Sepax Antibodix NP10 columns were used (Sepax, Newark, DE), with dimensions of 250 mm × 4.6 mm, 10 μm.

2.3. Mobile phase preparation

Appropriate amounts of buffer were dissolved in deionized water to produce a 2× concentrated solution, i.e., 19.2 mM Tris base, 12.0 mM piperazine, and 22.0 mM imidazole. Once dissolved, the solution was split into two equal aliquots. Each aliquot was diluted to 90% of the required final volume with deionized water. Each aliquot was then titrated to the appropriate pH by the addition of 10 N sodium hydroxide as necessary. Once titrated, deionized water was added to bring the solutions to the required volume. The mobile phases were then individually filtered through a 0.2 μm nylon filter prior to use. Mobile phases prepared for this work contained 9.6 mM Tris base, 6.0 mM piperazine and 11.0 mM imidazole, with pH values of either pH 6.0 (mobile phase A) or pH 9.5 (mobile phase B), unless otherwise indicated. This buffer composition is a modified piperazine/imidazole/tris buffer system originally reported by Kang and Frey [24] and used for mAb analysis by Farnan and Moreno [23].

2.4. Chromatographic conditions

Samples were diluted to a target protein concentration of 1 mg mL^{-1} in mobile phase A prior to placement into the autosampler. Samples in the autosampler were kept at a temperature of 5 ± 3 °C. Columns were placed in the column oven and the temperature control feature was employed to keep the oven temperature within a narrow range (±1 °C) from the set point during the studies.